AWARD NUMBER:	W81XWH-12-1-0236
TITLE:	EF5 PET of Tumor Hypoxia: A Predictive Imaging Biomarker of Response to Stereotactic Ablative Radiotherapy (SABR) for Early Lung Cancer
PRINCIPAL INVESTIGATOR:	Billy W Loo Jr, MD PhD
CONTRACTING ORGANIZATION:	The Leland Stanford Junior University Stanford CA 94305-2004
REPORT DATE:	November 2017
TYPE OF REPORT:	FINAL
PREPARED FOR:	U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
November 2017	**Final**	15 Aug 2012 - 14 Aug 2017

4. TITLE AND SUBTITLE

EF5 PET of Tumor Hypoxia: A Predictive Imaging Biomarker of Response to Stereotactic Ablative Radiotherapy (SABR) for Early Lung Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0236

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Billy W Loo Jr, MD PhD
875 Blake Wilbur Drive, Stanford CA 94305-5847

E-Mail: BWLoo@Stanford.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Leland Stanford Junior University
450 Serra Mall
Stanford, CA 94305-2004

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Stereotactic ablative radiotherapy (SABR) has become a new standard of care for early stage lung cancer in patients who are not candidates for surgery because of excessive surgical risk, and will be an important treatment option for a growing segment of patients with lung cancer. This is particularly true as lung cancer screening efforts are expected to diagnose a greater proportion of lung cancers at earlier stages, yet the aging of the population will lead to a greater proportion of patients having comorbidities that increase surgical risk. Tumor hypoxia is a major known mechanism of radiation resistance and is especially expected to affect very short courses of radiation as in SABR. Imaging using a third-generation hypoxia PET agent, 18F-EF5, is a promising approach for noninvasive hypoxia measurement that needs to be validated in the clinical setting.

Our objectives were (1) to understand the prevalence of hypoxia detectable by imaging in early stage NSCLC; (2) to validate 18F-EF5 PET as an indicator of tumor oxygenation status in this patient population; and (3) to evaluate 18F-EF5 PET as a prognostic imaging biomarker for local primary tumor control after SABR.

We enrolled a total of 15 patients with early stage lung cancer treated with SABR. The prevalence of hypoxia based on EF5-PET was 33%. Interventions intended to modify tumor hypoxia did not significantly alter EF5 uptake by tumors. There was a correlation between hypoxia on EF5-PET imaging and worse local tumor control, regional recurrence, distant metastasis, and overall survival, but these did not meet statistical significance in this small patient cohort. In conclusion, a substantial proportion of early stage lung tumors have hypoxia detectable by EF5-PET imaging, and these pilot results suggest the potential of EF5-PET as a biomarker of response of early stage lung cancer to SABR.

15. SUBJECT TERMS
Early stage lung cancer; Stereotactic ablative radiotherapy; Tumor hypoxia; EF5 PET imaging

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Contract Number: W81XWH-12-1-0236

Title: EF5 PET of Tumor Hypoxia: A Predictive Imaging Biomarker of Response to Stereotactic Ablative Radiotherapy (SABR) for Early Lung Cancer

Principal Investigator: Billy W Loo Jr, MD PhD

Introduction: Stereotactic ablative radiotherapy (SABR) has become a new standard of care for early stage lung cancer in patients who are not candidates for surgery because of excessive surgical risk, and will be an important treatment option for a growing segment of patients with lung cancer. This is particularly true as lung cancer screening efforts are expected to diagnose a greater proportion of lung cancers at earlier stages, yet the aging of the population will lead to a greater proportion of patients having comorbidities that increase surgical risk. Tumor hypoxia is a major known mechanism of radiation resistance and is especially expected to affect very short courses of radiation as in SABR. Imaging using a third-generation hypoxia PET agent, 18F-EF5, is a promising approach for noninvasive hypoxia measurement that needs to be validated in the clinical setting.

Our objectives are (1) to understand the prevalence of hypoxia detectable by imaging in early stage NSCLC; (2) to validate 18F-EF5 PET as an indicator of tumor oxygenation status in this patient population; and (3) to evaluate 18F-EF5 PET as a prognostic imaging biomarker for local primary tumor control after SABR. If accomplished, these would lay the foundation for future prospective therapeutic clinical trials using 18F-EF5 PET as a stratification factor, and ultimately to individualize therapy.

Keywords: Early stage lung cancer; Stereotactic ablative radiotherapy; Tumor hypoxia; EF5 PET imaging

Accomplishments:

What were the major goals of the project?

Four tasks were specified in the statement of work as follows:

Task 0: Pre-award preparation, including application for Stanford IRB and SRC approval and recruitment of clinical research coordinator – COMPLETED

Task 1: Patient recruitment – COMPLETED. Original target accrual of 43 patients was not met (see below). Accrual stopped for feasibility after 15 patients, who form the final cohort for analysis.
Task 2: Patient follow up – COMPLETED. Planned follow up of 18 months for all patients was exceeded. Minimum follow up in surviving patients was 22 months, and no patients were lost to follow up among the 15 patients accrued.
Task 3: Data analysis – COMPLETED. Results summarized below.
Task 4: Publication of results – ONGOING. A manuscript reporting outcomes of this study is being prepared for publication as described below.

What was accomplished under these goals?

Background and Objectives:

Tumor hypoxia is an important mechanism of resistance to radiation therapy, yet there are currently no clinically practical assays for tumor hypoxia in patients. PET imaging using tracers that accumulate in hypoxic tumors, such as EF5, has promise as a non-invasive biomarker for hypoxia and therefore tumor response to radiation therapy, particularly stereotactic ablative radiotherapy (SABR), which has emerged as a new treatment paradigm in early stage lung cancer. This project was a pilot clinical study of EF5-PET imaging as a potential imaging biomarker of response of early stage lung cancer to SABR. Eligible patients had biopsy proven early stage lung cancer planned to be treated with SABR.

There were three Specific Aims:
Aim 1 (Primary aim): To estimate the proportion of early stage lung cancers with tumor hypoxia as determined by EF5-PET (imageable hypoxia). EF5-PET imaging was performed in patients prior to SABR. The hypoxic fraction (the proportion of the tumor volume with detectable hypoxia) was defined as the proportion of image voxels within the tumor that had EF5 uptake exceeding that of the 95th percentile of EF5 uptake in normal background blood pool (measured in the aorta). Tumors with a hypoxic fraction greater than 10% were considered to have imageable hypoxia. In

this patient cohort, we determined the proportion of tumors that had imageable hypoxia at baseline.

Aim 2: To determine if interventions intended to modify tumor hypoxia alter the EF5 uptake. All patients had a second EF5-PET scan prior to SABR, but after a metabolic intervention to modify tumor hypoxia. Those patients who had imageable tumor hypoxia on the first EF5-PET scan breathed an oxygen-enriched gas (carbogen), which is expected to decrease tumor hypoxia transiently, immediately prior to the second EF5-PET scan to determine if tumor uptake of EF5 would be reduced. Patients who did not have imageable tumor hypoxia on the first EF5-PET scan received a dose of oral dichloroacetate (DCA), which is expected to increase tumor oxygen metabolism and therefore increase tumor hypoxia transiently, immediately prior to the second EF5-PET scan to determine if the tumor uptake of EF5 would be increased.

Aim 3: To determine if imageable hypoxia at baseline correlates with adverse clinical outcomes. The primary endpoint was local tumor control at 18 months. Secondary endpoints included regional tumor recurrence, distant metastasis, and overall survival. Patients were followed for these clinical endpoints.

Major findings/Results:

A total of 15 patients with early stage lung cancer were enrolled. All patients successfully completed the two EF5-PET scans, before and after the intervention to modify tumor hypoxia, and then underwent SABR. All patients had complete follow up until death or the time of analysis. The minimum follow up in surviving patients was 22 months.

Aim 1 (Primary aim): Five of 15 tumors had a hypoxic fraction greater than or equal to 10%. ***Thus the proportion of early stage lung tumors with imageable hypoxia is estimated to be 33%.***

Aim 2: Response by tumor EF5 uptake on PET imaging to hypoxia modifying interventions – Six patients with increased EF5 tumor uptake on the baseline EF5-PET scan received carbogen breathing prior to the second EF5-PET scan. Five of the six patients had a tumor hypoxic fraction greater than or equal to 10% at baseline, while one had a hypoxic fraction just under 10%. Figure 1 shows the tumor hypoxic fraction on EF5-PET imaging at baseline and after carbogen breathing.

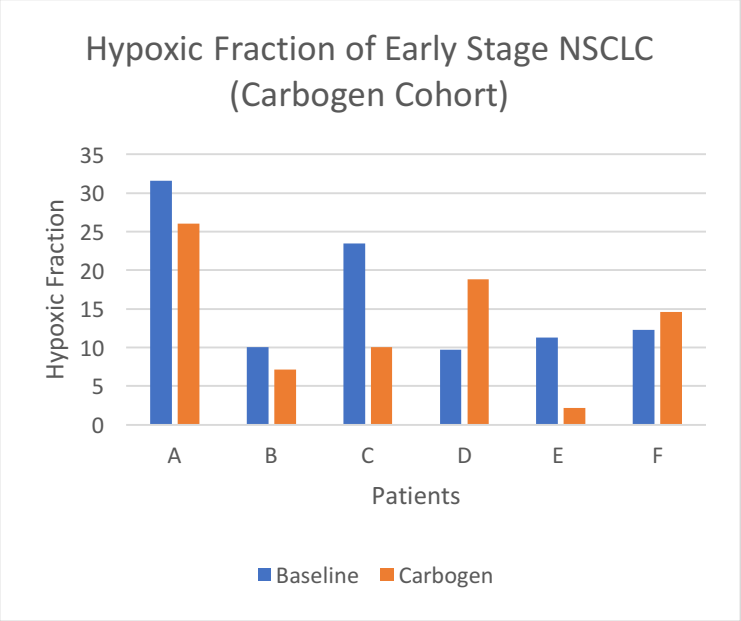

Figure 1: Effect of carbogen breathing on hypoxic fraction

There was no statistically significant reduction of tumor hypoxic fraction in response to carbogen breathing ($p = 0.56$).

Nine patients without increased EF5 tumor uptake on the baseline EF5-PET scan received oral DCA prior to the second EF5-PET scan. All 9 patients had a tumor hypoxic fraction substantially less than 10% (0-3.8%) at baseline. Figure 2 shows the tumor hypoxic fraction on EF5-PET imaging at baseline and after oral DCA.

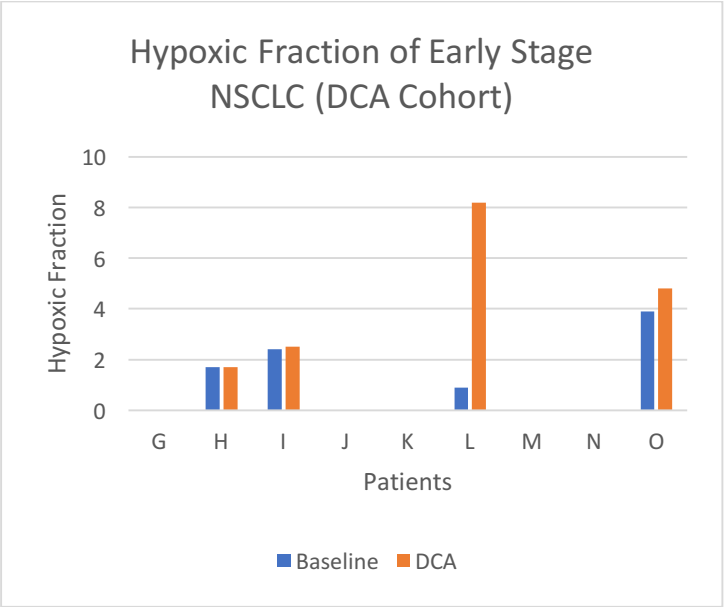

Figure 2: Effect of oral DCA on hypoxic fraction

There was no statistically significant increase in tumor hypoxic fraction in response to oral DCA (p = 0.25).

Thus, interventions intended to modify tumor hypoxia transiently did not significantly alter tumor EF5 uptake on PET imaging.

Aim 3: Correlation of tumor hypoxic fraction by EF5-PET imaging with clinical outcomes.

Figure 3 shows the cumulative incidence of local tumor failure (primary clinical outcome) after SABR in patients with and without imageable hypoxia, with death as a competing factor. Local tumor failure at 18 months was 20% in patients with imageable hypoxia compared to 0% in patients without imageable hypoxia, though this did not reach statistical significance (p = 0.14).

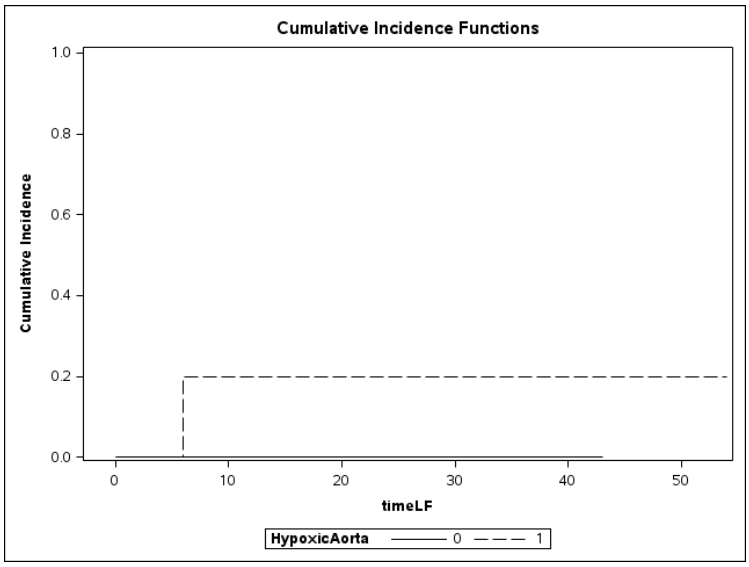

Figure 3: Cumulative incidence of local tumor failure after SABR

Figure 4 shows the cumulative incidence of regional recurrence after SABR in patients with and without imageable hypoxia, with death as a competing factor. Regional recurrence at 18 months was 20% in patients with imageable hypoxia compared to 9% in patients without imageable hypoxia, though this did not reach statistical significance (p = 0.16).

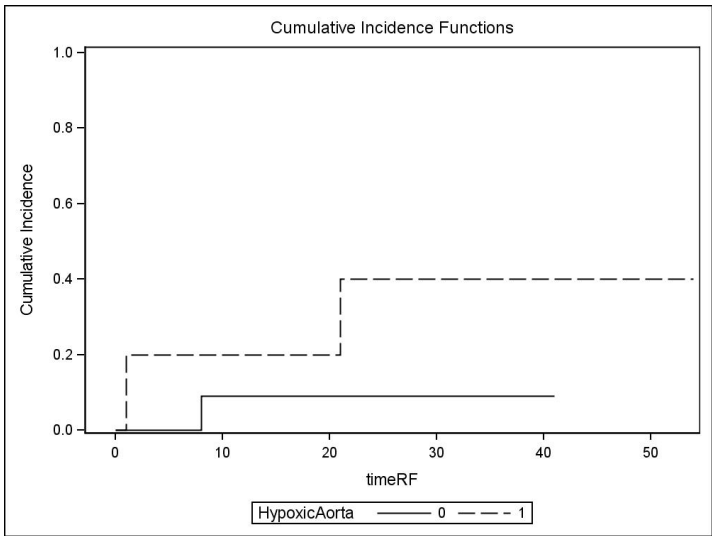

Figure 4: Cumulative incidence of regional recurrence after SABR

Figure 5 shows the cumulative incidence of distant metastasis after SABR in patients with and without imageable hypoxia, with death as a competing factor. Distant metastasis at 18 months was 40% in patients with imageable hypoxia compared to 9% in patients without imageable hypoxia, though this did not reach statistical significance (p = 0.16).

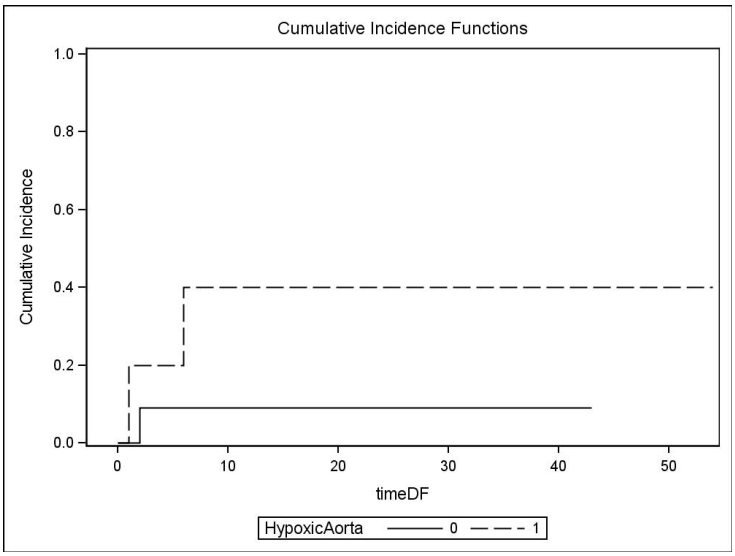

Figure 5: Cumulative incidence of distant metastasis after SABR

Figure 6 shows overall survival after SABR in patients with and without imageable hypoxia. Median survival time was 40 months in patients with imageable hypoxia compared to not reached in patients without imageable hypoxia, though this did not reach statistical significance (p = 0.53).

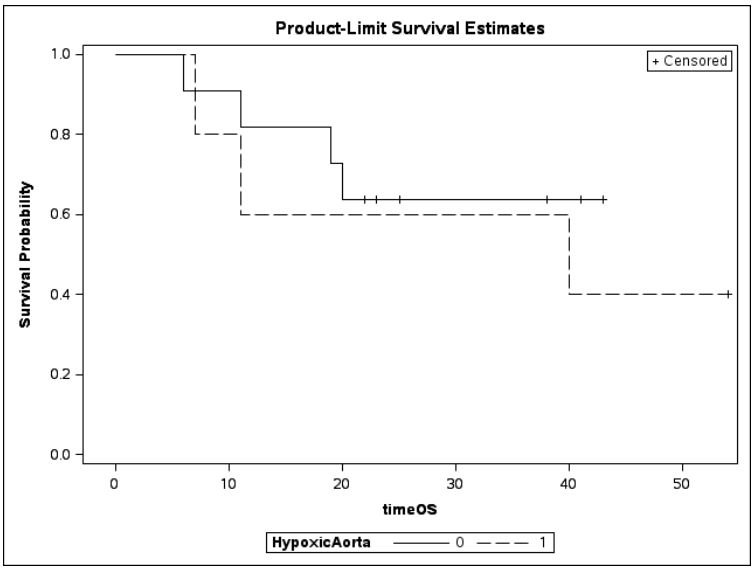

Figure 6: Overall survival after SABR

Imageable hypoxia on EF5-PET was associated with worse outcomes on all clinical endpoints studied (local tumor failure, regional recurrence, distant metastasis, and overall survival), though these differences did not reach statistical significance in this small patient cohort likely owing to inadequate power.

Conclusions:

A substantial proportion of early stage lung tumors have hypoxia detectable by EF5-PET imaging. These pilot results suggest the potential of EF5-PET as a biomarker of response of early stage lung cancer to SABR.

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report currently. As described below, we are now preparing a manuscript for publication to report the findings of this research project.

What do you plan to do during the next reporting period to accomplish the goals?

*This is the final report. We are now preparing a manuscript reporting the findings of this research project for submission to a special edition issue of the International Journal of Radiation Oncology*Biology*Physics on Imaging in Radiation Oncology (http://www.redjournal.org/call_for_papers). The submission deadline is December 1, 2017. This will fulfill the final task (Task 4: Publication of results) on the Statement of Work.*

Impact:

What was the impact on the development of the principal discipline(s) of the project?

The principal discipline of this project is radiation therapy for lung cancer. In particular, stereotactic ablative radiotherapy (SABR) has emerged as an important non-invasive treatment with curative potential for early stage lung cancer, particularly in patients who are not favorable candidates for surgery. Low oxygen levels in tumors (hypoxia) can compromise the effectiveness of radiation therapy, yet there is currently no clinically practical way to measure it. PET imaging using tracers that accumulate in hypoxic tumors hold promise as a non-invasive assessment of tumor hypoxia, and may help determine which patients may need a modified treatment strategy.

This study provides pilot results that suggest that a substantial proportion of early stage lung tumors have tumor hypoxia that can be detected by EF5-PET imaging. The results also suggest that EF5-PET imaging has promise to predict which tumors are more likely to recur after SABR, and may require additional therapy such as drug therapies. This warrants further study of this imaging modality.

What was the impact on other disciplines?

The results of this project will potentially also impact the discipline of diagnostic imaging. PET imaging using the tracer FDG, which accumulates in tissues with high glucose metabolism including many cancers, has revolutionized the staging of lung cancer and many other cancers and become a standard of care. However, few other

PET imaging tracers have entered routine clinical care. Tracers that probe aspects of tumor metabolism other than glucose use have the potential to provide clinically useful information to guide therapy. This pilot study suggests the potential of EF5-PET to predict treatment outcomes after SABR for early stage lung cancer, and possibly help individualize therapy for selected patients.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

Changes/Problems:

Changes in approach and reasons for change

Nothing to Report with respect to the scientific approach. However, as described below, a smaller number of patients than planned were accrued.

Actual or anticipated problems or delays and actions or plans to resolve them

The key challenge encountered in this project is that we were unable to complete accrual of the originally planned cohort of patients for this imaging study. The originally planned accrual was 43 patients (allowing for attrition to ensure 40 analyzable patients). The final accrual was 15 patients, all of whom are analyzable for the endpoints.

Several factors contributed to incomplete accrual. The major factor was that the logistical burden of performing two investigational EF5-PET scans in each patient, which had to be performed on separate days, led to much fewer patients agreeing to participate than anticipated, despite screening of the projected number of eligible patients in our clinics. The majority of patients in the demographic of our institutional catchment area travel a substantial distance for treatment, and extra visits not directly related to their treatment posed too great a burden on them. We attempted to streamline scheduling to reduce the total number of visits, which improved accrual but insufficiently to complete the originally planned accrual target. Other challenges included an initial delay in receiving the approval memorandum from DoD, and clinical research staff turnover leading to staffing shortages.

Per correspondence with Science Officer Dr. Elizabeth Yu, we determined that it would not be feasible to complete the originally targeted accrual, and that the 15 patients accrued would form the final cohort for analysis.

Changes that had a significant impact on expenditures

Because of accrual of a smaller patient cohort than originally planned, not all of the originally budgeted funds were expended. Unexpended funds will be returned to DoD as described in the final Financial Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

Products:

Publications, conference papers, and presentations

Journal publications. *Nothing to report currently. We are now preparing a manuscript reporting the findings of this research project for submission to a special edition issue of the International Journal of Radiation Oncology*Biology*Physics on Imaging in Radiation Oncology (http://www.redjournal.org/call_for_papers). The submission deadline is December 1, 2017.*

Books or other non-periodical, one-time publications. *Nothing to Report*

Other publications, conference papers, and presentations. *Nothing to Report*

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

A technique that has resulted from this research is an image analysis procedure for quantifying the hypoxic fraction of lung tumors that potentially correlates with clinical outcomes. Specifically, we define the hypoxic fraction to be the proportion of voxels in

a tumor exceeding an 18F-EF5 uptake threshold of the 95th percentile of uptake in the normal blood pool (in the aorta). This methodology will be shared in the manuscript currently being prepared for publication as described above.

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

Participants & Other Collaborating Organizations:

What individuals have worked on the project?

Name:	*Billy W Loo Jr*
Project Role:	*PI*
Nearest person month worked:	*5*
Contribution to Project:	*Principal investigator, patient recruitment, image and data analysis*

Name:	*Edward E Graves*
Project Role:	*Co-investigator*
Nearest person month worked:	*1*
Contribution to Project:	*Expertise on hypoxia imaging and imaging agent synthesis, image analysis*

Name:	*Gregory King*
Project Role:	*Postdoctoral scholar*
Nearest person month worked:	*8*
Contribution to Project:	*Software methodology development for image analysis*

Name:	*Frederick Lartey*
Project Role:	*Postdoctoral scholar*
Nearest person month worked:	*2*
Contribution to Project:	*Software methodology development for image analysis*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

- **Organization Name:** *Varian Medical Systems (commercial firm)*
- **Location of Organization:** *Palo Alto, CA*
- **Partner's contribution to the project:**

  In-kind support: *Provided the precursor for synthesizing the 18F-EF5 PET imaging agent*